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ARTICLE

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Differential metabolic effects of oral butyrate treatment in lean versus metabolic syndrome subjects

KEC Bouter¹, GJ Bakker¹, E. Levin¹, AV Hartstra¹, RS Kootte¹, SD Udayappan¹, S. Katiraei², L. Bahler¹, P. W. Gilijamse¹, V. Tremaroli³, M. Stahlman³, F. Holleman⁴, N. A. W. van Riel¹, HJ Verberne⁵, JA Romijn⁴, GM Dallinga-Thie¹, MJ Serlie⁶, MT Ackermans⁷, EM Kemper⁸, K. Willems van Dijk², F. Backhed³, AK Groen^{1,9} and M. Nieuwdorp^{1,3,4,10,11}

Abstract

Background: Gut microbiota-derived short-chain fatty acids (SCFAs) have been associated with beneficial metabolic effects. However, the direct effect of oral butyrate on metabolic parameters in humans has never been studied. In this first in men pilot study, we thus treated both lean and metabolic syndrome male subjects with oral sodium butyrate and investigated the effect on metabolism.

Methods: Healthy lean males ($n = 9$) and metabolic syndrome males ($n = 10$) were treated with oral 4 g of sodium butyrate daily for 4 weeks. Before and after treatment, insulin sensitivity was determined by a two-step hyperinsulinemic euglycemic clamp using [6,6-²H₂]-glucose. Brown adipose tissue (BAT) uptake of glucose was visualized using ¹⁸F-FDG PET-CT. Fecal SCFA and bile acid concentrations as well as microbiota composition were determined before and after treatment.

Results: Oral butyrate had no effect on plasma and fecal butyrate levels after treatment, but did alter other SCFAs in both plasma and feces. Moreover, only in healthy lean subjects a significant improvement was observed in both peripheral (median Rd: from 71 to 82 $\mu\text{mol/kg min}$, $p < 0.05$) and hepatic insulin sensitivity (EGP suppression from 75 to 82% $p < 0.05$). Although BAT activity was significantly higher at baseline in lean (SUVmax: 12.4 ± 1.8) compared with metabolic syndrome subjects (SUVmax: 0.3 ± 0.8 , $p < 0.01$), no significant effect following butyrate treatment on BAT was observed in either group (SUVmax lean to 13.3 ± 2.4 versus metabolic syndrome subjects to 1.2 ± 4.1).

Conclusions: Oral butyrate treatment beneficially affects glucose metabolism in lean but not metabolic syndrome subjects, presumably due to an altered SCFA handling in insulin-resistant subjects. Although preliminary, these first in men findings argue against oral butyrate supplementation as treatment for glucose regulation in human subjects with type 2 diabetes mellitus.

Introduction

As a steep increase in the prevalence of obesity is seen in the Western world, with expectations rising up to about 33% of obese adults that will develop insulin resistance and ultimately type 2 diabetes mellitus, novel insights in this epidemic disease are necessary^{1,2}. Unfortunately, current therapeutic strategies can only partly prevent the complications associated with insulin resistance, notably

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micro- and macrovascular diseases, and therefore new treatment modalities are urgently needed³. Recent animal studies have suggested that intestinal microbiota might play a metabolic role in weight control and insulin sensitivity⁴. Elevated generation of the short-chain fatty acids (SCFAs) butyrate, acetate, and propionate by bacterial fermentation of dietary fibers is thought to contribute to systemic energy regulation by decreasing hepatic glucose and lipid production⁵. Concomitantly, SCFAs can activate intestinal G protein-coupled receptors, including GPR 41 and GPR 43, which also improves metabolism⁶. Recent data however suggest that depending on metabolic background these SCFAs might have opposite effects. On the one hand, rodents on a high-fat diet that develop diet-induced obesity (DIO) have increased (gut microbiota-driven) acetate production with concomitant insulin resistance⁷. On the other hand, oral supplementation of butyrate in DIO rodents led to an increase of insulin sensitivity and energy expenditure⁸.

Although the exact roles of SCFAs in metabolism are only partly understood⁵, recent animal data have suggested that regulation of brown adipose tissue (BAT) by the SCFA butyrate^{9,10} as well as bile acids via FGF19¹¹ can improve insulin sensitivity and lipid parameters in rodent models of metabolic disease. The mitochondria in brown adipocytes contain uncoupling protein-1, which inhibits ATP synthesis at the expense of heat¹². Therefore, enhanced BAT activity is important for energy expenditure and is considered to be of influence on insulin sensitivity^{13,14}. Since BAT activation has been reported in DIO insulin-resistant mice that were treated with the oral SCFA butyrate⁸, we performed a human pilot study to investigate the effect of 1 month of daily oral sodium butyrate treatment on hepatic and peripheral insulin sensitivity (as assessed by 2H2-glucose-based two-step hyperinsulinemic normoglycemic clamp), resting energy expenditure (REE), and BAT activity in both lean and obese insulin-resistant males. Based on available animal data we performed a pilot trial that oral butyrate treatment would improve insulin sensitivity via activation of BAT in both lean and metabolic syndrome subjects. We however found that this intervention was not effective in human metabolic syndrome.

Methods

Caucasian, healthy, lean (body mass index (BMI) 20–25 kg/m²) males and Caucasian obese males (BMI > 25 kg/m²) were recruited via local newspaper advertisements. Participants had to be >18 years old. Those obese subjects fulfilling the National Cholesterol Education Program (NCEP) criteria for metabolic syndrome ($\geq 3/5$: fasting plasma glucose ≥ 5.6 mmol/l; triglycerides (TG) ≥ 1.7 mmol/l; waist circumference > 102 cm; high-density lipoprotein cholesterol (HDLc) < 1.03 mmol/l; blood pressure $\geq 130/85$ mm Hg; and BMI ≥ 30 kg/m²) were

included¹⁵. Exclusion criteria for both groups were a history of a cardiovascular event, cholecystectomy, use of any medication known to influence gut microbial composition (e.g., proton pump inhibitors, antibiotics, and pro-/pre-/synbiotics) in the last 3 months as well as medication influencing metabolism (e.g., lipid-lowering, antidiabetic, and/or antihypertensive drugs). All subjects had a stable weight and dietary intake for 3 months prior to inclusion. Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board (IRB) and conducted at the Academic Medical Center (AMC) Amsterdam, The Netherlands, in accordance with the Declaration of Helsinki. All authors had access to the study data, reviewed, and approved the final manuscript.

Upon inclusion during the winter season (since brown fat can be measured most reliably during that season)¹⁴, subjects were admitted to the clinical trial unit at the AMC for a baseline visit (visit 1 + 2) and then started treatment with 4 g (2 g BID) of sodium butyrate supplementation (Sensilab, Poland), which was the maximum daily dose allowed by IRB based on a previous human intervention study¹⁶. Compliance was evaluated by counting the number of capsules returned after 4 weeks of treatment. Measurements performed at baseline were repeated in all subjects after 4 weeks (visit 3 + 4) (see supplemental Fig. 1). Participants were asked to maintain their habitual physical activity pattern, but to refrain from heavy exercise in the days preceding the hyperinsulinemic euglycemic clamp. Participants were encouraged to continue their usual diet. All participants filled out an online nutritional diary (www.dieet-wijzer.nl) to monitor caloric intake of carbohydrates, fat, protein, and fibers before and after 4 weeks of butyrate treatment.

Hyperinsulinemic euglycemic clamp

After an overnight fast, REE using indirect calorimetry was determined. Then, hepatic and peripheral glucose metabolism was measured at baseline during a two-step hyperinsulinemic euglycemic clamp using [6,6-²H₂]-glucose to measure endogenous glucose production (EGP) and hepatic and peripheral insulin sensitivity (rate of glucose disposal (Rd)) as previously described¹⁷. EGP and the peripheral uptake of glucose (Rd) were calculated using modified versions of the Steele equations for the non-steady state¹⁸ and were expressed as $\mu\text{mol/kg/min}$.

BAT activity

BAT activity was determined as described before¹⁹. In short, all subjects were tested during the winter season after an overnight fast. They were exposed to mild cold (16 °C–17 °C) in an air-cooled room for 2 h. During the cold exposure, subjects were wearing underwear only. After 1 h of cold exposure, the radioactive tracer ¹⁸F-FDG was administered, adjusted for BMI, leading to dosages of

200 MBq; ^{18}F -FDG positron emission tomography-computed tomography (PET-CT) was performed directly after the 2-h cold exposure (i.e., 1 h after ^{18}F -FDG administration). We measured standardized maximal uptake (SUVmax), mean uptake (SUVmean), and volume of BAT. Each BAT volume was measured using a ^{18}F -FDG threshold-based delineation. Therefore, volumes measured reflect activated BAT volume. All visually identified areas with active BAT were included in the analysis. All analyses were done by a single person (L.B.).

Biochemistry

Fasting glucose (Hitachi), insulin (Diagnostic products), C-reactive protein (Roche, Switzerland), free fatty acid (FFA; Wako), and lipopolysaccharide-binding protein (LBP, HyCult) were determined in fasted plasma samples. Total cholesterol, low-density lipoprotein cholesterol (LDLc), HDLc, and TG were determined in EDTA-containing plasma using commercially available enzymatic assays (Randox, Antrim, UK and DiaSys). All analyses were performed using a Selectra autoanalyzer (Sopachem, The Netherlands). FFA concentrations were determined with an enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals, Neuss, Germany). Insulin was determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA). Fasting glucagon was determined with the Linco 125I RIA (Linco Research, St Charles, MO, USA). FGF19 was determined by a commercial enzyme-linked immunosorbent assay (Quantikine Human FGF-19 Immunoassay). Fasting plasma bile acid profiles were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described²⁰. On the last 2 days of the week before the start and 4 weeks after oral butyrate treatment, subjects were asked to collect 24 h feces (stored at 4 °C) for fecal bile acid composition using gas chromatography as described²¹, including the primary bile acids cholic acid and chenodeoxycholic acid as well as the secondary bile acids deoxycholic acid, lithocholic acid (LCA), urso-deoxycholic acid, and iso-LCA. The total amount of primary and secondary bile salts was calculated as the sum of the individually quantified bile salts²². SCFA concentrations (acetate, butyrate, and propionate) and lactate were determined in overnight-fasted EDTA plasma and in fresh morning fecal samples using LC-MS/MS²³.

Fecal microbiota analyses

Total genomic DNA was isolated from feces as previously described²³. Fecal microbiota composition was profiled by sequencing the V4 region of the 16S rRNA gene on an Illumina MiSeq instrument (Illumina RTA v1.17.28; MCS v2.5) with 515F and 806R primers designed for dual indexing and the V2 Illumina kit (2 × 250 bp paired-end reads). 16S rRNA genes from each sample

were amplified in duplicate reactions in volumes of 25 μL containing 1× Five Prime Hot Master Mix (5 PRIME GmbH), 200 nM of each primer, 0.4 mg/mL bovine serum albumin, 5% dimethylsulfoxide, and 20 ng of genomic DNA. PCR was carried out under the following conditions: initial denaturation for 3 min at 94 °C, followed by 25 cycles of denaturation for 45 s at 94 °C; annealing for 60 s at 52 °C and elongation for 90 s at 72 °C; and a final elongation step for 10 min at 72 °C. Duplicates were combined, purified with the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel), and quantified using the Quant-iT PicoGreen dsDNA kit (Invitrogen). Purified PCR products were diluted to 10 ng/ μL and pooled in equal amounts. The pooled amplicons were purified again using Ampure magnetic purification beads (Agencourt) to remove short amplification products. Illumina reads were merged using PEAR²⁴ and filtered by removing all reads that had at least one base with a *q*-score lower than 20. Final reads were analyzed with the software package QIIME (version 1.8.0). Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using an open-reference OTU-picking approach with UCLUST against the Greengenes reference database (13_8 release)²⁵. All sequences that failed to cluster when tested against the Greengenes database were used as input for picking OTUs de novo. Representative sequences for the OTUs were Greengenes reference sequences or cluster seeds, and were taxonomically assigned using the Greengenes taxonomy and the Ribosomal Database Project Classifier²⁶. Representative OTUs were aligned using PyNAST and used to build a phylogenetic tree with FastTree which was used to calculate α - and β -diversity of samples using Phylogenetic Diversity and UniFrac²⁷. Three-dimensional principal coordinate analysis (PCoA) plots were visualized using Emperor²⁸. Chimeric sequences were identified with ChimeraSlayer²⁹ and excluded from all downstream analyses. Similarly, OTUs that could not be aligned with PyNAST, singletons, and low-abundant OTUs with a relative abundance < 0.002% were also excluded. To correct for differences in sequencing depth, a same amount of sequence was randomly sub-sampled from each sample (30 000 sequences/sample); therefore, a total of 1 886 870 sequences and 1181 OTUs were included in the diversity analyses.

Statistical analyses

Differences in clinical variables between lean and Met-Syn subjects were tested with unpaired *T*-test or Mann–Whitney test depending on normality of the data. Moreover, paired *T*-test or Wilcoxon test were used for differences within groups. Linear correlations between SCFAs were calculated using Spearman's correlation coefficients. A *p*-value < 0.05 was considered significant. With regard to fecal microbiota analyses, relative OTU

abundances were calculated as previously described²³. Subsequently, significantly dissimilarities in gut microbiota composition between individuals before and after treatment were assessed with the Bray–Curtis (β -diversity) index calculated at the OTU and at the genus level by Mann–Whitney test. The Bray–Curtis dissimilarities were plotted using unconstrained PCoA allowing to visualize the dimensions explaining most variability in the

dissimilarity matrix and tested using multivariate non-parametric analysis of variance³⁰. Moreover, to study whether specific OTUs were discriminating between lean and MetSyn groups before and after treatment we used elastic net algorithm modeling as recently published^{31,32}. In short, a randomization test was conducted to test the statistical validity of the results obtained with the elastic net algorithm. The dataset containing information on assignment of the subjects to lean or MetSyn group was randomly reshuffled while the corresponding microbial profiles were kept intact. This was repeated multiple times and the area under receiver-operating characteristic (ROC) curve (AUC) scores (see supplemental Fig. 2) were generated each time by application of the elastic net algorithm to the permuted data. As a test statistic, the ROC AUC score was chosen with the null hypothesis that there is no difference in microbial profile between the confirmed metabolic syndrome and lean groups. Correlation plots were made using R statistical software. Multivariate statistical modeling was done using Numerical Python and MATLAB.

Results

A total of 20 male subjects (10 healthy lean males and 10 males with metabolic syndrome) were included. One lean subject was excluded from the analyses due to technical difficulties with clamp and PET-CT. Therefore, 19 subjects were available for analysis (Table 1). As expected, baseline age, BMI, REE, blood pressure, fasting lipids, glucose, and insulin were significantly different between lean controls and metabolic syndrome subjects. Following butyrate treatment, a significant increase in plasma total cholesterol and LDLc was observed only in the metabolic syndrome group (Table 1).

Effect of oral sodium butyrate treatment on plasma and fecal SCFAs

At baseline, subjects with metabolic syndrome showed a different composition of plasma SCFA, with a lower percentage of acetate and higher percentages of propionate and butyrate (Table 2a) compared to the lean

Table 1 Characteristics of study subjects

	Lean group (N = 9)		Metabolic syndrome group (N = 10)	
	Before	After	Before	After
Age (years)	25 ± 2.4		42 ± 2.4 [#]	
Body mass index (kg/m ²)	22.1 ± 2.4	21.9 ± 2.0	33.2 ± 3.6 [#]	33.1 ± 3.6
Systolic blood pressure	129 ± 9	121 ± 13	139 ± 16	146 ± 24
Diastolic blood pressure	74 ± 7	69 ± 9	82 ± 7*	79 ± 16
Heart rate (bpm)	64 ± 11	62 ± 10	66 ± 6	64 ± 7
Cholesterol (mmol/l)	4.2 ± 0.8	4.2 ± 0.8	4.9 ± 0.7*	5.2 ± 0.8 [^]
HDLc (mmol/l)	1.4 ± 0.2	1.4 ± 0.3	1.0 ± 0.2 [#]	1.1 ± 0.2
LDLc (mmol/l)	2.2 ± 0.5	2.2 ± 0.5	2.8 ± 0.5*	3.0 ± 0.5 [^]
TG (mmol/l)	0.8 ± 0.3	0.7 ± 0.2	1.8 ± 0.3 [#]	1.7 ± 0.2
FFA (mmol/l)	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
REE (kcal/day)	1728 ± 157	1776 ± 200	1938 ± 214	1854 ± 322
Energy intake (kcal/day)	1884 ± 705	1753 ± 520	1916 ± 425	1966 ± 324

Values are expressed as means ± SD

Differences between lean and metsyn subjects were tested with unpaired T-test or Mann–Whitney test based on Gaussian distribution (* $p < 0.05$, [#] $p < 0.01$). Moreover, paired T-test or Wilcoxon test was used for differences within groups ([^] $p < 0.05$)

HDLc high-density lipoprotein cholesterol, LDLc low-density lipoprotein cholesterol, TG triglycerides, REE resting energy expenditure

Table 2a SCFA concentrations in plasma before and after oral butyrate supplementation

Group	Time point	Total SCFA (μM)	Acetate (μM)	Acetate (%)	Propionate (μM)	Propionate (%)	Butyrate (μM)	Butyrate (%)
Lean	0	117.7 ± 44.7	106.1 ± 42.9	89.6 ± 3.6	8.5 ± 3.3	7.6 ± 3.1	3.1 ± 0.9	2.8 ± 0.7
Lean	4w	115.2 ± 40.5	103.7 ± 36.2	90.2 ± 2.8	7.3 ± 3.8	6.3 ± 2.3	4.3 ± 3.4	3.5 ± 1.8
MetSyn	0	79.9 ± 68.6	68.6 ± 20.7	85.6 ± 2.8*	8.2 ± 2.7	10.4 ± 2.8*	3.2 ± 1.0	4.0 ± 0.8**
MetSyn	4w	67.7 ± 28.2	59.0 ± 25.5	86.9 ± 5.0	5.6 ± 2.6 [#]	8.6 ± 3.9	3.1 ± 2.8	4.5 ± 3.2

Short-chain fatty acid (SCFA) concentrations in plasma were measured before and after butyrate supplementation in healthy lean males (Lean) and obese insulin-resistant males (MetSyn). Data are presented as mean ± standard deviation

* Represents a statistically significant difference at baseline between the Lean and MetSyn groups, * $p < 0.05$; ** $p < 0.01$. [#] Represents a statistically significant difference between time point 0 and time point 4w (4 weeks), $p < 0.05$

Table 2b SCFA concentrations in feces before and after oral butyrate supplementation

Group	Time point	Total SCFA (μmol/g)	Acetate (μmol/g)	Acetate (%)	Propionate (μmol/g)	Propionate (%)	Butyrate (μmol/g)	Butyrate (%)
Lean	0	326.7 ± 226.5	206.8 ± 153.3	62.4 ± 3.0	69.3 ± 64.5	7.6 ± 3.1	50.7 ± 20.7	17.6 ± 5.6
Lean	4w	191.3 ± 82.8	118.8 ± 50.3	63.0 ± 10.4	41.1 ± 23.3	6.3 ± 2.3	31.4 ± 26.3	16.2 ± 8.4
MetSyn	0	340.6 ± 141.9	208.3 ± 97.6	60.1 ± 7.9	78.3 ± 36.9	10.4 ± 2.8	54.0 ± 23.9	16.2 ± 4.1
MetSyn	4w	226.9 ± 129.0 ^{##}	139.8 ± 85.3 [#]	61.9 ± 9.7	44.5 ± 25.9 ^{##}	8.6 ± 3.9	42.5 ± 34.8 [#]	17.9 ± 8.5

Short-chain fatty acid (SCFA) concentrations in feces were measured before and after butyrate supplementation in healthy lean males (Lean) and obese insulin-resistant males (MetSyn). Data are presented as mean ± standard deviation

[#] Represents a statistically significant difference between time point 0 and time point 4w (4 weeks), [#] $p < 0.05$; ^{##} $p < 0.01$

subjects. There were no baseline differences in fecal SCFA concentrations between the two groups (Table 2b). In contrast, 4 weeks of oral butyrate supplementation affected the fecal SCFA concentrations to a greater extent than plasma levels. Whereas the plasma propionate concentration was significantly decreased in the MetSyn group after butyrate treatment compared to baseline, in feces we found a significant reduction of total SCFA, acetate, propionate, and butyrate concentrations in the MetSyn group after 4-week butyrate supplementation. Finally, fasting plasma lactate levels were increased at baseline in insulin-resistant subjects compared to lean subjects, but remained largely unaffected upon butyrate treatment in both groups (metabolic syndrome: from 0.6 ± 0.1 to 0.6 ± 0.1 versus lean: from 0.4 ± 0.2 to 0.4 ± 0.1 mmol/l), whereas fecal concentrations of lactate were not altered upon butyrate treatment (lean: from 1.1 ± 0.6 to 1.1 ± 0.3 versus insulin resistant: from 1.4 ± 0.5 to 1.6 ± 0.9 μmol/g feces, ns).

Effects of oral sodium butyrate on glucose metabolism

As expected, metabolic syndrome subjects were characterized by markedly impaired hepatic and peripheral insulin sensitivity as compared to healthy lean control subjects (Fig. 1). In lean healthy males, there was a significant improvement in peripheral insulin sensitivity (Rd: from 71 ± 10 to 82 ± 16 μmol/kg min, $p < 0.05$) after 4 weeks' oral butyrate while no change was observed in the metabolic syndrome subjects (Rd: from 33 ± 10 to 31 ± 9 μmol/kg min, ns; see Fig. 1a and supplemental Table 1). In line, a significant improvement in hepatic insulin sensitivity (expressed as % EGP suppression) was observed in lean healthy males (from 75 ± 7 to $82 \pm 8\%$, $p < 0.05$), while no effect was observed in the metabolic syndrome group (from 60 ± 7 to $58 \pm 11\%$, ns; Fig. 1b and supplemental Table 1).

Effects of oral sodium butyrate on BAT activation and bile acid metabolism

In line with previous reports^{13,14,20}, BAT activity at baseline was significantly higher in lean subjects

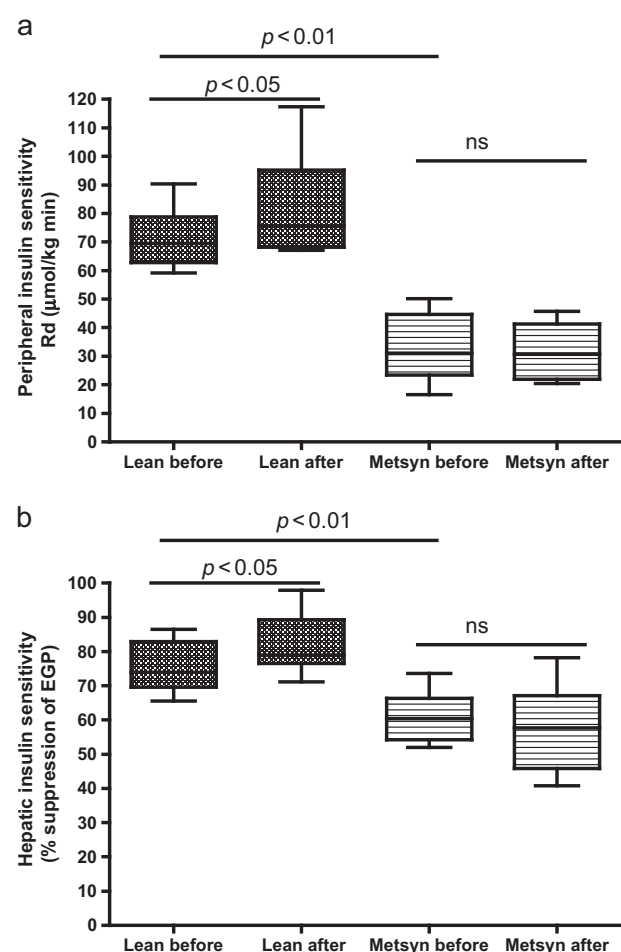


Fig. 1 Effect of oral sodium butyrate on peripheral insulin sensitivity and hepatic insulin sensitivity in both lean and metabolic syndrome subjects. The effect of oral butyrate treatment in lean subjects and metabolic syndrome subjects on **a** peripheral (Rd or glucose rate of disappearance) and **b** hepatic insulin sensitivity (% suppression of EGP). Data are presented as median plus interquartile range. Differences between lean and MetSyn subjects were tested with Mann–Whitney test and Wilcoxon test for differences within groups

(SUVmax: 12.4 ± 1.8) when compared to metabolic syndrome subjects (SUVmax lean: 0.3 ± 0.8 , $p < 0.01$). Upon 4 weeks of oral sodium butyrate supplementation BAT

activation was not affected in either lean healthy males (SUVmax: from 12.4 ± 1.8 to 13.3 ± 2.4) or in metabolic syndrome subjects (SUVmax: from 0.3 ± 0.8 to 1.2 ± 4.1 , ns). With respect to bile acid metabolism fasting plasma levels of FGF19 were significantly higher at baseline, in lean (FGF19: 133 ± 64 pg/mL) than in MetSyn subjects (FGF19: 88 ± 36 pg/mL, $p < 0.05$). In line, lean subjects were characterized by lower plasma primary and secondary bile acids in both plasma and feces when compared to MetSyn subjects (see supplemental table 2). However in both groups, oral butyrate treatment had neither an effect on FGF19 levels (lean: to 123 ± 64 and MetSyn: to 66 ± 31 pg/mL, ns) nor on primary and secondary bile acids in both plasma and 24 h feces (see Supplementary table 2).

Effects of oral sodium butyrate on fecal microbiota and clinical correlations

At baseline no significant difference was observed in bacterial (β) diversity between lean and metabolic syndrome subjects; also oral butyrate treatment had no significant effect (Simpsons diversity index lean: from 0.97 ± 0.02 to 0.97 ± 0.02 versus MetSyn: from 0.95 ± 0.01 to 0.95 ± 0.02 , ns; Fig. 2a, b). Using multivariate analyses, we did not observe an effect of oral butyrate treatment on overall fecal microbiota composition at 4 weeks in both lean and MetSyn subjects. However, when applying our recently published elastic net algorithm^{31,32} to identify the intestinal bacterial species most discriminative between lean and MetSyn groups following oral butyrate treatment (Fig. 2c) we could with high sensitivity (AUC 0.88, also see supplemental Fig. 2) identify *Lachnospiraceae* and *Bacteroides* (in lean) and *Coriobacteriaceae* and *Clostridiales cluster XIVa* (in metabolic syndrome) to be the most significantly affected by butyrate treatment ($p < 0.05$).

Subsequent correlation analyses revealed that in lean subjects these *Bacteroides* species in fecal samples were significantly correlated with both pre- and posttreatment plasma butyrate levels (Fig. 3a). However in metabolic syndrome subjects, *Coriobacteriaceae* species in fecal samples were significantly correlated with both pre- and posttreatment hepatic insulin sensitivity (suppressed EGP), whereas an inverse significant correlation with plasma LDLc and butyrate levels was seen (Fig. 3b). Finally, in lean subjects fecal acetate levels were significantly correlated with fecal butyrate and propionate levels in both pre- and post oral butyrate treatment feces, whereas these correlations were not found in metabolic syndrome subjects.

Discussion

Recent observational studies have supported a role for decreased intestinal SCFA butyrate-producing bacterial strains in human insulin resistance^{5,23}, yet interventional

studies are lacking. To our knowledge this is the first human pilot study studying oral butyrate supplementation on human glucose and BAT metabolism. Although we were unable to detect any increases in butyrate concentration in the feces or plasma after 4 weeks of treatment, other SCFAs were differentially altered between both treatment groups. Moreover and in contrast to lean subjects, we observed that oral butyrate supplementation did not result in improved glucose metabolism in the metabolic syndrome subjects, who would be the most logical treatment group.

The SCFAs, acetate, propionate, and butyrate make up the majority of SCFAs and are present within the colonic lumen in a molar ratio of 3:1:1, predominantly generated by fermentation of dietary fibers⁵. Whereas the proximal colon has a function in saccharolytic fermentation (e.g., generation of SCFAs), the distal colon mainly drives proteolytic fermentation (e.g., generation of p-cresol)⁵. In a recent study systemic availability of SCFA in healthy humans was shown to be 36%, 9%, and only 2% for acetate, propionate, and butyrate, respectively³³. In line with other human data using rectal infusions of SCFA^{34–36}, intestinally produced SCFA propionate is a known preferred precursor for gluconeogenesis, whereas acetate and butyrate are involved in regulation of cholesterol synthesis, a correlation that is also found in our study (Fig. 3)³⁷. Once SCFAs have been generated in the colon, they are subjected to a high intestinal inter-conversion in the intestine of healthy subjects³⁸. Unfortunately, we did not observe large changes in butyrate concentrations in the feces or plasma after 4 weeks of supplementation, most likely because most of the butyrate is very quickly utilized as an energy source by intestinal epithelium. Nevertheless, other SCFA levels were altered and correlated with markers of insulin sensitivity in metabolic syndrome patients (Fig. 3b) suggesting different handling and flux of these SCFAs in the obese insulin-resistant state. In support of this hypothesis, a peroxisome proliferator-activated receptor γ -dependent switch from hepatic lipid synthesis to increased energy utilization was seen only in obese (DIO) mice^{8,39}. In line with Fig. 3, altered SCFA substrate preference has been previously shown in healthy lean subjects^{33,40–43} in whom colonic SCFA propionate is used as a gluconeogenic substrate while concomitantly inhibiting the utilization of acetate for cholesterol synthesis^{44,45}. Moreover, in line with tracer data derived from rodent studies more than half a century ago⁴⁶, we speculate that intestinally produced SCFA are differentially handled in the obese insulin-resistant state in order to regulate glucose and lipid metabolism⁴⁵. In contrast with animal work⁴⁷, we observed no relation between butyrate supplementation and bile acid metabolism. Although further research is needed, we did find some associations between metabolic parameters including SCFA levels and

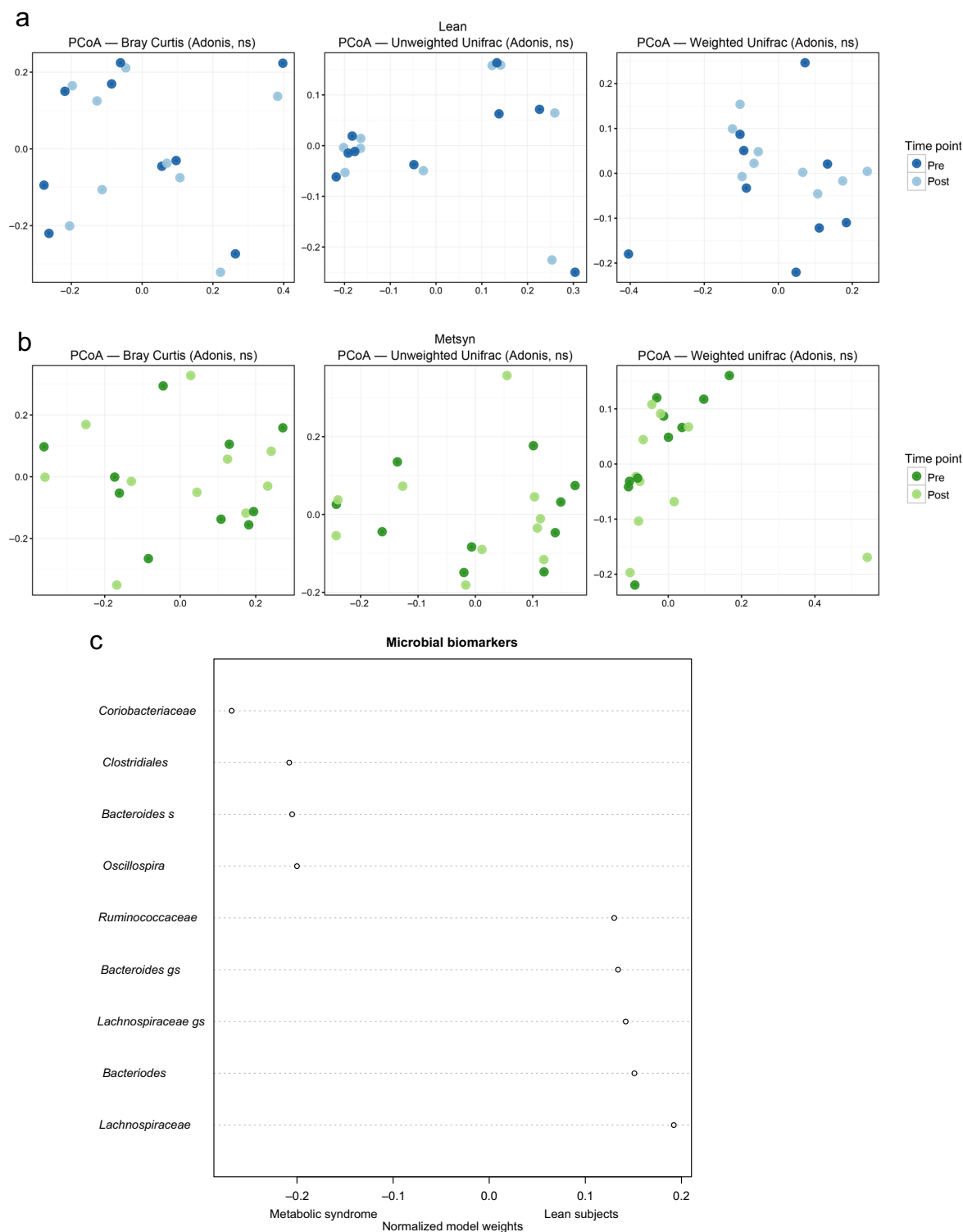


Fig. 2 Effect of oral sodium butyrate on microbiota composition in both lean and metabolic syndrome subjects. Changes in fecal microbiota composition before and after butyrate in either **a** lean (dark and light blue dots, respectively) or **b** MetSyn subjects (dark and light green dots, respectively) as depicted by PCA biplots based on Bray–Curtis (left panel), unweighted unifrac (middle panel), and weighted unifrac (right panel). **c** Significant associations between changes in fecal bacterial strains (depicted on the y-axis) between lean and metabolic syndrome (depicted on x-axis) after 4 weeks of butyrate treatment; a positive weight represents an association with butyrate treatment in lean subjects, whereas a negative weight represents an association with butyrate treatment in metabolic syndrome subjects. The higher the weight, the stronger the association

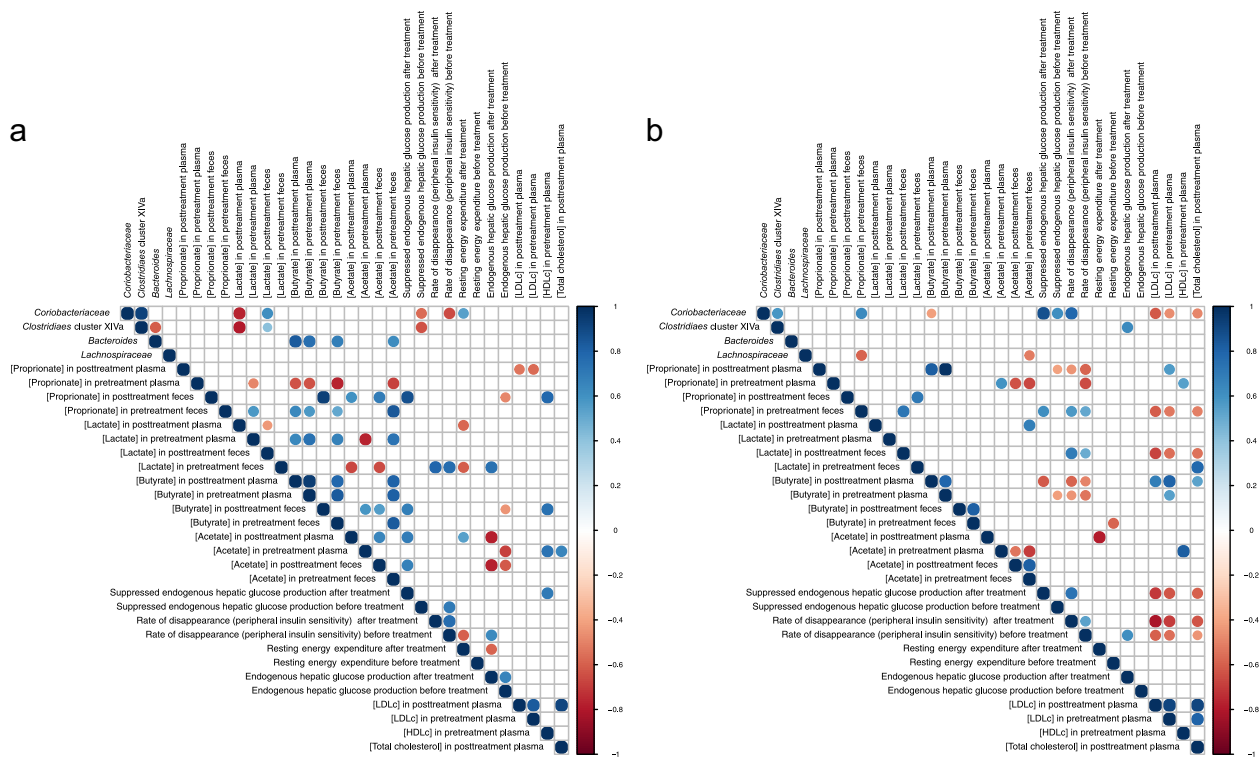


Fig. 3 Correlation plots showing effect of oral butyrate on other biochemistry markers in both lean and metabolic syndrome subjects.

Correlation plots of fecal SCA, specific fecal bacterial strains, and clinical markers in **a** lean subjects and **b** insulin-resistant metabolic syndrome subjects. Only significant correlations after correction for multiple comparisons ($p < 0.05$) are depicted. Blue depicts positive correlation, whereas red color means inverse correlation. The size and strength of the color depict the magnitude of the correlation

specific intestinal bacterial strains (Fig. 3) suggesting a potential role of specific intestinal bacterial strains^{48,49} in this altered SCFA handling between healthy and insulin-resistant human subjects.

Our study has certain limitations. First, our findings are derived from a small pilot study of both healthy lean and obese insulin-resistant male Caucasian subjects in whom butyrate was released in the small intestine rather than in the colon⁵⁰. As metabolic syndrome takes several years to develop¹, subjects with metabolic syndrome were significantly older than lean controls. Second, the butyrate dose used was lower than the 5% of bodyweight quantities of butyrate usually given in mouse studies^{8,37}. Based on previous literature¹⁶, we had to keep a maximum daily oral dose of 4 g/day. At this used amount, peripheral insulin sensitivity improved only in the lean despite similar dose and compliance in both lean and metabolic syndrome groups. As we did not adjust our daily dose of oral butyrate as amount/kg bodyweight in our study, this might have also resulted in a sub-therapeutic dose of sodium butyrate in the more obese metabolic syndrome subjects. Thus, larger follow-up (placebo) controlled trials with different dose ranges are needed in different degrees of insulin resistance (e.g., metabolic syndrome versus overt type 2 diabetes). Moreover, as SCFA levels in feces and

plasma are hard to measure reliably due to their volatile nature, future human studies combining oral with intravenously administered stable isotope-labeled SCFA are warranted to accurately monitor fluxes³³ in order to validate our findings and to further unravel SCFA production and catabolism in relation to differences in microbiome function using a larger group of healthy and insulin-resistant subjects. Future research will thus have to elucidate whether (short versus long term) treatment with either oral SCFA or administration of specific butyrate-producing intestinal bacteria has any therapeutic potential in insulin-resistant subjects.

Disclaimer

There are no patents, products in development, or marketed products to declare in relation to this study.

Study Highlights

What is current knowledge?

Short-chain fatty acids (SCFAs) are produced by intestinal microbiota.

Animal models have shown that SCFA butyrate improves glucose metabolism.

SCFA butyrate treatment in animals increases brown adipose tissue activation.

What is new here?

Oral butyrate suppletion only beneficially affects glucose metabolism in lean subjects.

In contrast to animal models, no effect on brown adipose tissue was found in both lean and obese insulin-resistant subjects.

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Competing interests

Guarantor of the article M. Nieuwdorp.

Specific author contributions M.N., A.K.G., J.A.R., F.B., and K.W.v.D. designed the study. E.M.K. provided the GMP produced sodium butyrate capsules. K.E.C.B., G.J. B., L.O., R.S.K., A.V.H., P.W.G., S.D.U., S.K., L.H., H.J.V., and L.B. performed the research. V.T., M.S., F.H., H.J.V., N.A.W.v.R., M.T.A., M.J.S., and G.M.D.-T. provided analytic tools. K.E.C.B., E.L., and V.T. performed the statistical analysis. K.E.C.B., A.K.G., F.B., and M.N. drafted the paper. All authors critically reviewed the manuscript.

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